AD_____

Award Number: W81XWH-13-1-0117

TITLE: Effects of Pharmacologic and Genetic Inhibition of Alk on Cognitive Impairments in NF1 Mutant Mice

PRINCIPAL INVESTIGATOR: Jacob Raber, PhD

CONTRACTING ORGANIZATION: Oregon Health & Science University
Portland, OR 97201-3011

REPORT DATE: June 2015

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
June 2015	Annual Report	1 June 2014 – 31 May 2015

4. TITLE AND SUBTITLE

Effects of Pharmacologic and Genetic Inhibition of Alk on Cognitive Impairments in NF1 Mutant Mice

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-1-0117

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Jacob Raber, PhD, PI
Tessa Marzulla, Research Assistant Raber Laboratory

E-Mail: raberj@ohsu.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Oregon Health & Science University
3181 SW Sam Jackson Park Rd
Portland, Oregon 97239

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We studied the expression of Alk and the effects of Alk mutations on learning and memory in mice. Concordant with studies in flies, we found enhanced retention of spatial memory in Alk mutant mice. Retention of spatial memory is a hippocampal dependent function. We also demonstrated expression of Alk throughout the adult murine hippocampus. The behavioral phenotype of Alk mutant mice is the opposite of the behavioral phenotype of Nf1 mutant mice. We hypothesize that the genetic interaction between Alk and Nf1 in mice is similar to the behavioral phenotypes of Alk and Nf1 mutations in flies and that pharmacologic or genetic inhibition of Alk in Nf1 mutant mice will attenuate or even rescue learning impairments in mice. We describe the breeding data for the genetic study and the behavioral data so far for the genetic study and pharmacological study. The behavioral data are very encouraging and support our hypothesis. While slower due to the breeding effort involved, this project is going extremely well.

15. SUBJECT TERMS
cognitive performance, pharmacological inhibition, spatial memory

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	17	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Introduction

Nf1 mutations, which occur in approximately 90% of patients with neurofibromatosis, are associated with cognitive impairment. Impaired academic performance is common and often requires special education [1,2]. Mental retardation is seen in 6-7% of children, a percentage about three times higher than that in the general population. Specific learning disabilities in reading, spelling and math occur in 20% of children without overt central nervous system pathology [3]. Impairments in visual spatial processing and more complex executive functions have also been reported [4,5].

The mechanism underlying the cognitive impairments associated with neurofibromatosis is hard to study in humans. The phenotypes observed in mice indicate a specific function for Neurofibromin in the hippocampus [6]. The Morris water maze, an assay of murine hippocampus-dependent learning, has been employed to explore this phenotype [6-8]. In a specific test of the ability to recall the location of a non-visible, submerged platform based on remote visual cues, *Nf1* heterozygous mutant mice consistently show impaired spatial memory compared to wild-type controls. This phenotype is genetically and pharmacologically susceptible to modification by manipulation of Ras, a regulator of the MAP kinase (MAPK) signal transduction cascade [6].

One of Neurofibromin's biochemical functions is to modulate signal transduction through the Ras-MAPK pathway. Neurofibromin is a negative regulator of this pathway that catalyzes the conversion of activated Ras-GTP to inactive ras-GDP [7,9]. The Ras-MAPK signal transduction pathway is canonically responsive to receptor tyrosine kinase (RTK) activation. Recently, strong evidence has emerged from studies of Drosophila that activation of Anaplastic Lymphoma Kinase (Alk) by its ligand, Jelly belly (Jeb), is the physiologically relevant target of negative regulation by *Nf1* [10]. The link between Jeb/Alk signaling and Neurofibromin is supported by studies of *Nf1* regulation of body size and associative learning in flies. Based on the phenotypic resemblance between activation of Alk and inactivation of *Nf1* and genetic interactions between *Nf1* and *alk* in Drosophila, Gouzi et al. hypothesized that NF1 functions as a specific negative regulator of Alk signaling to enhance learning and memory. They showed that *Nf1* and *alk* mutations strongly interact with respect to both regulation of body size and olfactory associative learning. They also showed that the learning impairments in *Nf1* mutants could be corrected or rescued by genetic or pharmacologic inhibition of Alk. In support of the hypothesis that Neurofibromin acts directly downstream of Alk activation, they also demonstrated that expression of *Nf1* mRNA specifically in cells that also express *alk* was sufficient to rescue both body size and learning phenotypes of *Nf1* mutant flies [10]. This implies that Neurofibromin acts as a negative regulator of Alk activation and that inhibition of Alk is a potential therapeutic intervention to compensate for haplo-insufficiency of *Nf1.*

Alk was originally identified as a human proto-oncogene frequently activated by chromosomal translocation in lymphomas [11]. It plays a causative role in a number of other human malignancies, including non-small cell lung cancer and neuroblastoma [12-15]. Orally active small molecule inhibitors have shown notable effectiveness in the treatment of lung cancer and are actively being tested for the treatment of neuroblastoma [16-18].

The normal function of Alk in humans is less clear though its expression in both the developing and adult nervous system of mammals has focused recent investigations on behavioral phenotypes [19]. The hypothesis of behavioral and other neural functions of Alk in humans is supported by a variety of studies in model organisms, including Drosophila, C. elegans and mice.

Behavioral functions for an Alk ligand were first described in C. elegans. An unbiased, forward genetic screen for mutations that effect integration of conflicting sensory inputs identified Hen-1, the C. elegans homologue of Jeb, as a signal that participated in behavioral response to simultaneous, conflicting attractive and aversive stimuli [20]. One of the most striking findings was that the Hen-1/Jeb requirement was not developmental. The behavioral phenotype

could be rescued by a temporally controlled transgene only if it was expressed in adults. The behavioral phenotype of Hen-1/Jeb mutants included an effect on associative learning.

In Drosophila, Jeb and Alk were first characterized for their roles in early muscle development [21-23]. Investigation of their functions in the nervous system has required conditional genetic techniques to circumvent embryonic lethality. Several functions for Jeb and Alk have been established in the developing and adult Drosophila nervous system: 1) Jeb and Alk are essential for correct axon targeting of a subset of photoreceptors [24]; 2) Late embryonic maturation of the larval neuromuscular junction requires Jeb and Alk, though they are not required for axon targeting or synapse formation in this context [25]; 3) Jeb and Alk protect neurogenesis when developing larvae are nutritionally challenged [26]; and 4) Jeb and Alk have been implicated in associative learning and regulation of body size upstream of Nf1. Recently, Jeb activation of Alk in the larval neuromuscular junction, a glutamatergic synapse, was shown to be a potent negative regulator of synaptic transmission [27].

Key words

Cognitive performance, pharmacological inhibition, spatial memory

Overall project summary

We have studied the effects of Alk mutations on learning and memory in mice. Concordant with Gouzi et al's findings in flies, we find enhanced performance in retention of spatial memory in Alk mutant mice [28]. The behavioral phenotype of *Alk* mutant mice is the opposite of the behavioral phenotype found in *Nf1* mutant mice. Based on these data, we hypothesize that the genetic interaction between *Alk* and *Nf1* in mice is similar to the behavioral phenotypes of *alk* and *Nf1* mutations in flies. We further propose that pharmacologic and genetic inhibition of Alk in *Nf1* mutant mice will attenuate or even rescue learning defects in mice, as it does in flies.

The specific aim of our proposal is to test the effects of genetic and pharmacologic inhibition of Alk on retention of spatial memory in heterozygous *Nf1* mutant mice. If pharmacologic inhibition of Alk in mice rescues the learning defect of *Nf1* mutations this will provide the basis for pursuit of similar strategies in humans. We propose two approaches to test the strategy of inhibiting Alk to treat the cognitive impairment caused by heterozygous *Nf1* mutation in mice, one genetic the other pharmacologic. These two approaches are complimentary and will address potential weaknesses inherent to each separate approach.

Key Research Accomplishments

This project involves complicated breeding and two mutant mouse models that needed to be imported, after processing all the required paper work for it. Also, as the mice we requested needed to be bred and become available first, this delayed the shipment of these mice. More specifically, we received 4 male and 3 female Alk KO mice on the C57BL/6J background on 9/25/2013 from Dr. Liliana Attisano at the University of Toronto and 2 male NF1+/- mice, on the 129 genetic background, on 10/09/2013 from Dr. Nancy Ratner at the Cincinnati Children's Hospital. The complicated breeding scheme combined with the delayed shipment of the mice has prolonged this project and as a result we requested to continue and finish this project in Yr 3, which was approved earlier this year.

The breeding data for the genetic study are indicated in Table 1. We have had an extremely hard time obtaining one of our target genotypes, the Nf1-/+; Alk WT, from our current

double heterozygote breeding strategy. Therefore, we have decided to breed some of the mice from the vehicle-testing group of the pharmacological study in order to get the desired phenotype. The resulting pups will have the same genetic background (F2 B6/129Svj), be genotyped for Nf1, and will be tested at 3-4 months of age to boost our animal numbers. This breeding is ongoing and will continue in Yr 3.

Table 1. Breeding data for the genetic study.

Genetic Study			
Total Female	**Total Male**	**Non-Target Genotypes**	**Total**
1	2	unknown complete genotype (multiple failed PCR reactions)	3
8	15	Alk het, NF1 het	23
2	7	Alk het, WT	9
Total Female	**Total Male**	**Target Genotypes**	**Total (Goal= 8 mice per sex, per genotype)**
0	1	WT, NF1 het	1
8	11	WT, WT	19
12	18	Alk KO, NF1 het	30
11	4	Alk KO, WT	15

The breeding data for the genetic study are indicated in Table 2. The behavioral data for our pharmacological study are described in detail below. These data are very encouraging and suggest the Alk inhibitor has an effect on learning and memory. In order to complete our pharmacological study, we will be breeding for more Alk-/Alk- (Alk KO) to assure the specificity of the Alk inhibitor by demonstrating that it has no effects in Alk homozygous knockout mice. In Yr 3, we will cross untested Alk- heterozygotes from the genetic study to get enough Alk KOs to subsequently breed and obtain a cohort of Alk KOs that will be given the Alk inhibitor or vehicle and tested.

Table 2. Breeding data for the pharmacological study.

Pharmacological study		
Cohort 1	***Cohort 2***	**Total (Goal= 8 mice per sex, per genotype)**
DRUG	**DRUG**	
3 female Nf1 hets	3 female Nf1 hets	**6**
4 female WT	4 female WT	**8**
2 male Nf1 hets	5 male	**7**

	Nf1 hets	
5 male WT	3 male WT	**8**
VEHICLE	**VEHICLE**	
1 female Nf1 het	3 female Nf1 hets	**4**
5 female WT	3 female WT	**8**
3 male Nf1 hets	3 male Nf1 hets	**6**
3 male WT	4 male WT	**7**

SUMMARY OF ALL MICE BRED FOR ENTIRE PROJECT

Below we summarize the number of all mice bred so far for this project. Total number of mice bred from 2 founder males (Nf1-/+), 3 founder females (Alk KO) and 4 female B6 WT for the Alk inhibitor study= **372**

Genetic Experiment (6 cohorts):

F1 double heterozygotes used for breeding F2s=17

F1s not used for breeding=40

Tested F2 mice=101

Unused F2 mice due to death or undesired genotypes=141

F2 Alk-/+ currently breeding=8

F2 mice too young to test @ last cohort=7

Note: 10 total F1 breeding pairs (3 for cohorts 1 and 2, 7 for cohorts 3-6) to obtain all F2s

Alk Inhibitor Pharmacological Experiment (2 cohorts):

Tested mice=56

Unused mice due to cap in cohort #s=2

Note: 4 breeding pairs used to generate mice for the Alk inhibitor experiment.

Conclusions

In Yr 2 of this project, we continued and updated the breeding efforts for this project. The breeding effort is going well and we anticipate that we will not need to request additional mice for breeding, although we received way less mice than we would have used if we were able to purchase the mice or obtain more mice for breeding. The preliminary data obtained in the genetic and pharmacological studies are encouraging and support our overall hypothesis that reducing Alk in the context of NF1 improves cognitive performance and that not only genetic manipulation but also pharmacological treatment is effective. We are very excited about these preliminary data and look forward to finalize this project in the third year. Although less important clinically, we will also assess whether the Alk inhibitor acts through Alk by assessing cognitive performance in Alk deficient mice treated with the inhibitor.

Abstracts and Publications

Not yet

Reportable Outcomes

Alk-NF1 genetic study

In Table 3, the 88 mice we tested for the genetic study are indicated.

Label	Alk Genotype	NF1 Genotype	Females	Males	Total
WT	WT	WT	8	11	19
2xMut	Alk-/-	Nf1-/+	12	16	28
2xHet	Alk-/+	NF1-/+	10	15	25
HomAlk	Alk-/-	WT	11	4	15
NF1	WT	NF1	0	1	1

Below we describe the cognitive data. We would like to emphasize that more mice will be added to this study and more analyses will be performed. This is just a progress report of what we have done so far and have seen in terms of overall patterns of genetic effects. The mice were first tested in the water maze. The mice were trained to locate the platform in three

distinct locations during hidden platform training (Hidden Location 1: sessions 1-4; Hidden Location 2: sessions 5-6; Hidden Location 3: sessions 7-8). Subsequently, the mice were trained to locate a visible platform (Visible Location: sessions 9-10). The cumulative distance to the target location where the platform was is shown in Fig. 1. The data were analyzed as a repeated-measures ANOVA with genotype and sex as between group factors. For the first and third hidden platform and visible platform locations, there were no effects of genotype or sex or interactions. However, for the second platform location, there was a genotype x session interaction ($p = 0.048$), sex x session interaction ($p = 0.015$), and a trend towards a genotype x session x sex three way interaction ($p = 0.065$). When velocity was included as a covariate in the analysis, it was not significant for the second platform location and the interactions were maintained. When swim speeds were analyzed for the hidden platform location, there was an effect of genotype ($p = 0.015$). WT mice swam faster than 2xMut ($p = 0.013$) and HomAlk ($p = 0.037$) and there was a trend towards swimming faster than 2xHet ($p = 0.080$). During the visible platform training, there was also an effect of genotype on swim speeds ($p = 0.004$). Posthoc analysis indicated that there was a trend of WT mice swimming faster than 2xMut mice ($p = 0.053$).

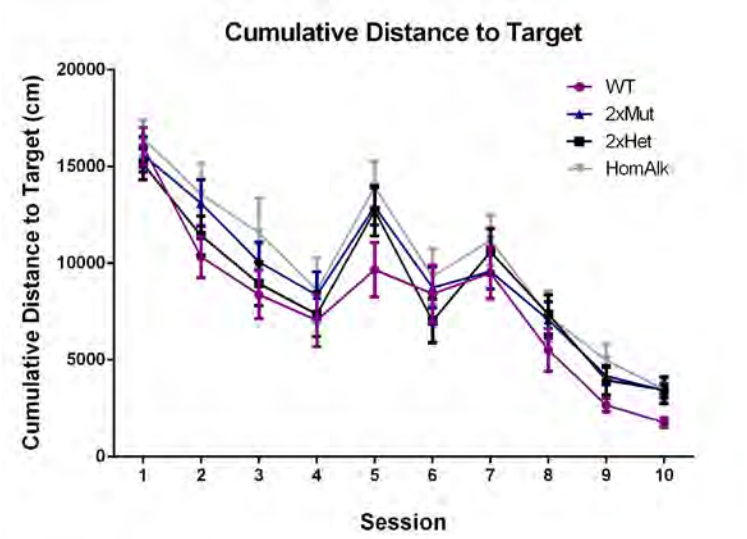

Fig. 1. Water maze learning curves. For details, see text.

The day after training to locate the first hidden platform location, the mice were tested for spatial memory retention in a probe trial (no platform) (Fig. 2). In females, 2xMut mice (target versus left: $p < 0.001$; target versus right: $p < 0.0001$) and HomAlk mice (target versus left or right: $p < 0.05$) spent more time searching in the target quadrant than the left and right quadrant but not the opposite quadrant. The better memory performance of the 2xMut than 2xHet female mice is consistent with our hypothesis that reducing Alk in the context of NF1 is beneficial for cognition. This genotype difference was not seen in male mice. Both 2xMut (target versus left: $p < 0.01$; target versus right: $p < 0.001$; target versus opposite: $p < 0.05$) and 2xHet (target versus left and opposite: $p < 0.05$; target versus right: $p < 0.01$) male mice showed spatial memory retention and spent more time searching in the target quadrant than any other quadrant. These

data suggest that female mice are more susceptible to effects of NF1 on spatial memory retention in the water maze.

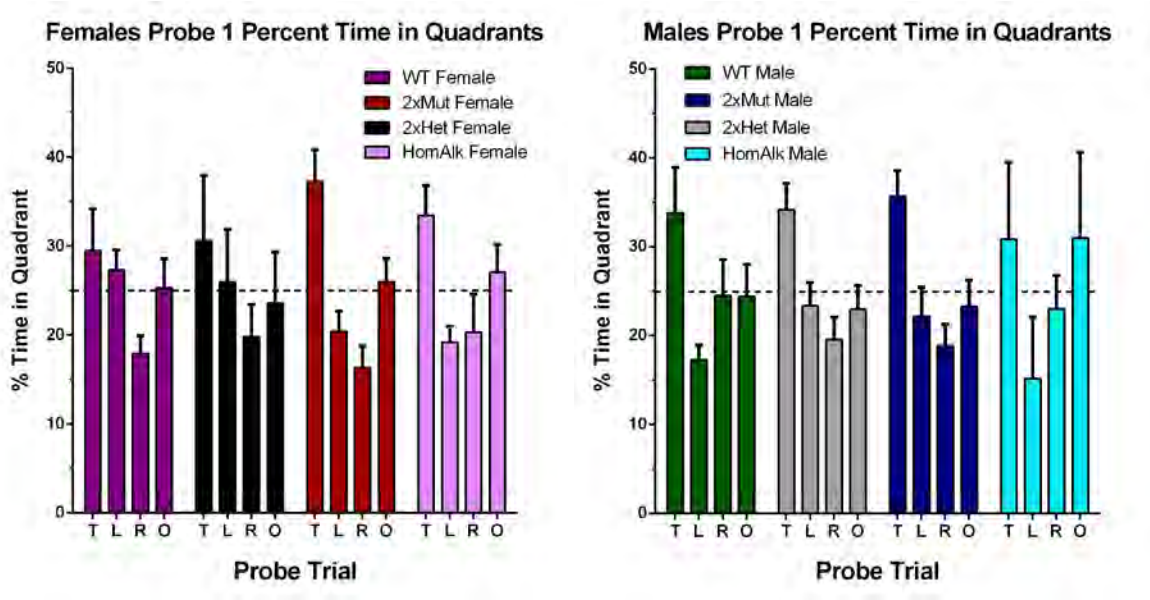

Fig. 2. Spatial memory retention in the water maze. For details, see text.

Next the mice were tested for acquisition and extinction of hippocampus-dependent contextual fear. During training (day 1), there was an effect of genotype on motion prior to the first tone (baseline motion, $p = 0.0029$, Fig. 3). WT mice moved more than 2xHet ($p < 0.01$) and 2xMut ($p < 0.05$) mice. There was no effect of genotype on freezing in response to the tone during training (not shown). There was a trend towards an effect of genotype on motion in response to the shock during training but that did not reach significance ($p = 0.08$). There was no effect of genotype on freezing between the tone-shock pairings during training (not shown), indicating that all genotypes acquired the task equally well.

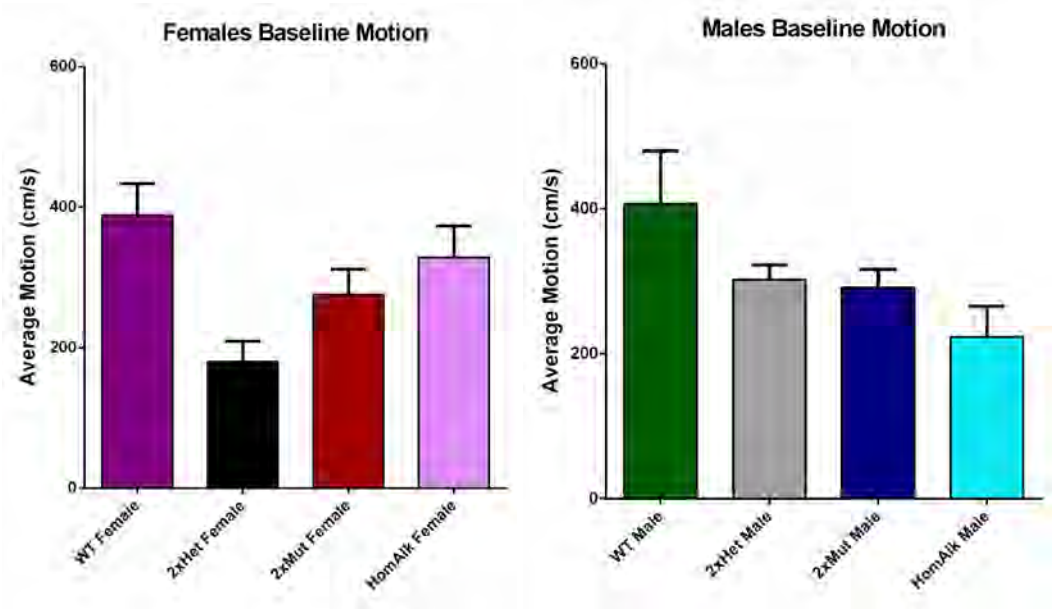

Fig. 3. Motion prior to the first tone in the contextual fear training task. For details, see text.

Next, we determined the ability of the mice to suppress the memory when placed daily in the same environment as that used for learning the tone-shock association. The data are shown in Fig. 4. There was a trend towards a genotype x sex interaction for extinction of contextual fear but that did not reach significance (p = 0.068). Using a multivariate analysis with genotype and sex as between group factors, there was a genotype x sex interaction on days 3 (p = 0.035) and day 7 (p = 0.045), trends towards a genotype x sex interaction on days 4 (p = 0.071) and 5 (p = 0.053) and a trend towards a genotype effect on day 1 (p = 0.076). In males, 2xMut mice showed lower freezing levels on all 7 days, while this genotype difference was only seen on day 1 in female mice. Consistent with this pattern, when a multivariate analysis was performed for female and male mice separately, there was an effect of genotype on day 3 (p = 0.041) and a trend towards an effect of genotype on day 4 (p = 0.075) and an effect of genotype on day 1 in females (p = 0.054).

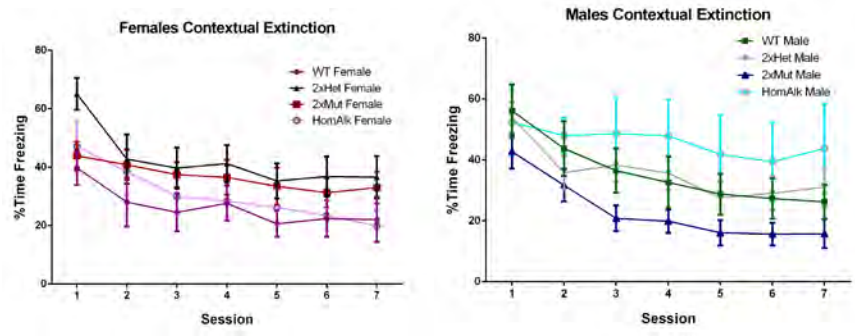

Fig. 4. Extinction of contextual fear. For details, see text.

Alk-NF1 pharmacological study

As indicated above for the genetic study, we are excited to share the very recent cognitive data based on the pharmacological study but would like to reiterate that animals will be added to this study and additional analyses will be performed. As a consequence, these data should be considered preliminary at this point. When all the hidden platform sessions were analyzed together, there was a genotype x sex interaction (p = 0.023) and a genotype x session interaction (p = 0.023) (Fig. 5). In addition, during training to locate the second hidden platform location, there was a trend towards an effect of genotype (p = 0.056). During training to locate the third hidden platform location, there was a treatment x session interaction (p = 0.008) and a genotype x treatment interaction (p = 0.036). Overall, the Alk inhibitor improved learning during the hidden platform training. No effects of the treatment were seen when the platform was visible, indicating that this effect is specific for spatial learning and memory and not related to task learning in general.

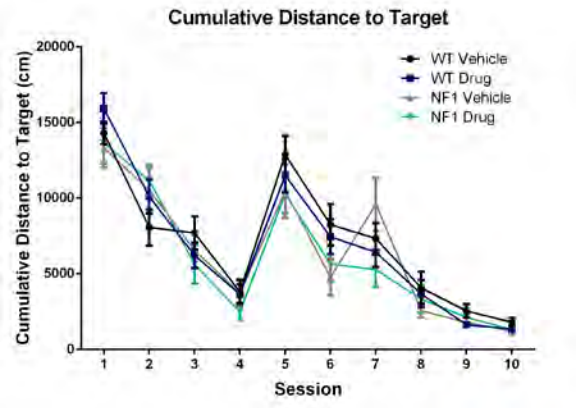

Fig. 5. Effects of Alk inhibitor on water maze learning curves. For details, see text.

Fig. 6 shows spatial memory retention 24 hours following the last training to locate the first hidden platform location. In female mice, the Alk inhibitor increased the time spent searching in the target quadrant in NF1 but not WT mice. This pattern was not seen in male mice. In addition, NF1 mutant mice did not show impairments in this probe trial. However, NF1 did show impairments in spatial memory retention in the probe trial following training to locate the second hidden platform location and this impairment was mitigated by the Alk inhibitor (Fig. 7).

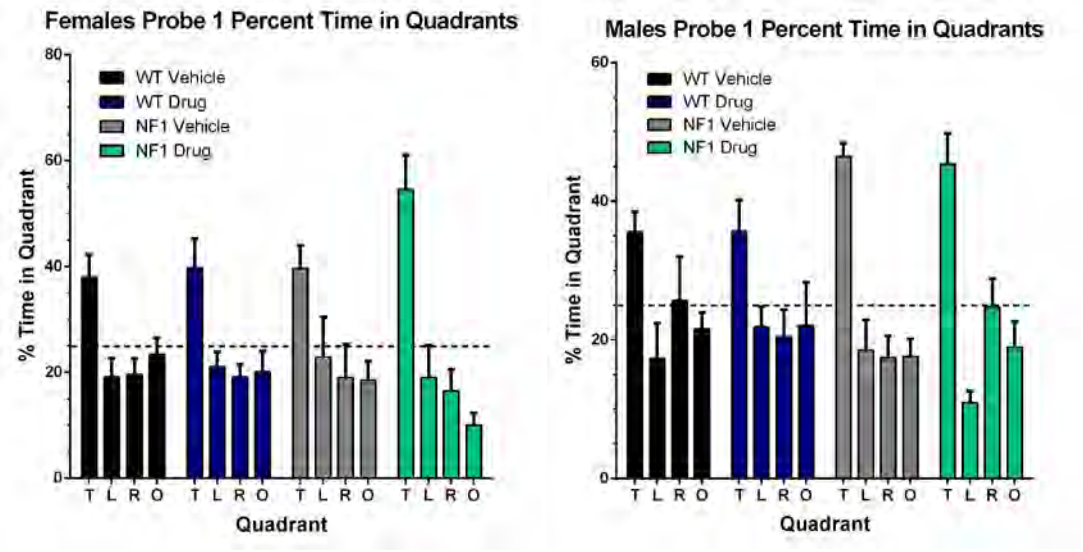

Fig. 6. Effects of Alk inhibitor on spatial memory retention following training to locate the first hidden platform in the water maze. For details, see text.

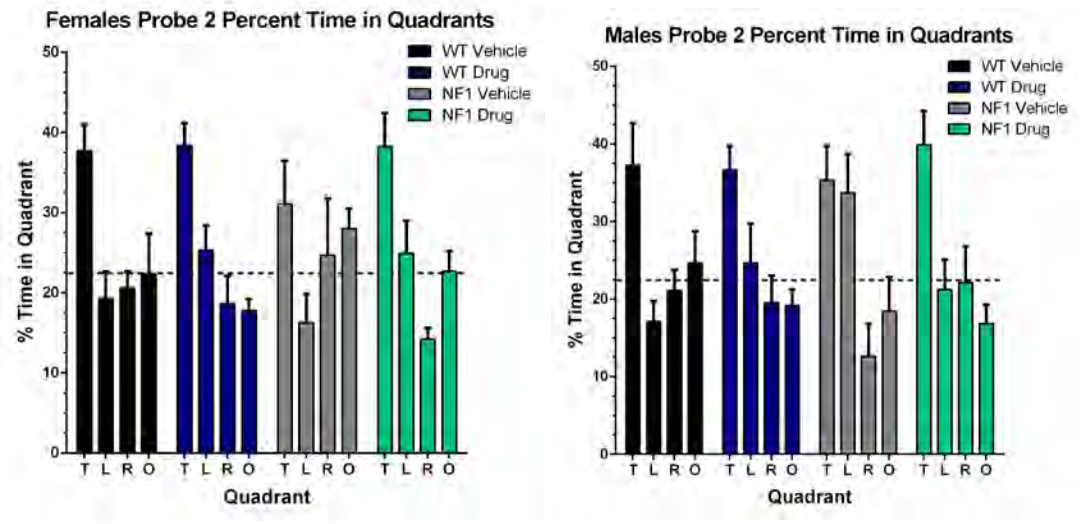

Fig. 7. Effects of Alk inhibitor on spatial memory retention following training to locate the second hidden platform in the water maze. For details, see text.

Finally, we assessed learning and extinction of contextual fear. There were no effects of genotype, treatment, or a genotype x treatment interaction for motion prior to the first tone-shock pairing during training (not shown). However, when response to the shock during the training was analyzed, there was an effect of genotype (p = 0.005) and an effect of sex (p = 0.012). NF1 mutant mice showed a stronger response to the shock than WT mice and males showed a stronger response to the shock than female mice. However, there were no effects of treatment or an interaction with treatment. When response to the tone during training was analyzed, there was an effect of sex (p = 0.029) and a tone x sex interaction (p = 0.005) but no effect of treatment. In general, the female mice showed a stronger response to the tone than the males. However, an effect of treatment (p = 0.016) was seen when freezing between the tone-

shock pairings was analyzed on the training day (Fig. 8). The Alk inhibitor reduced freezing as compared to vehicle treated mice.

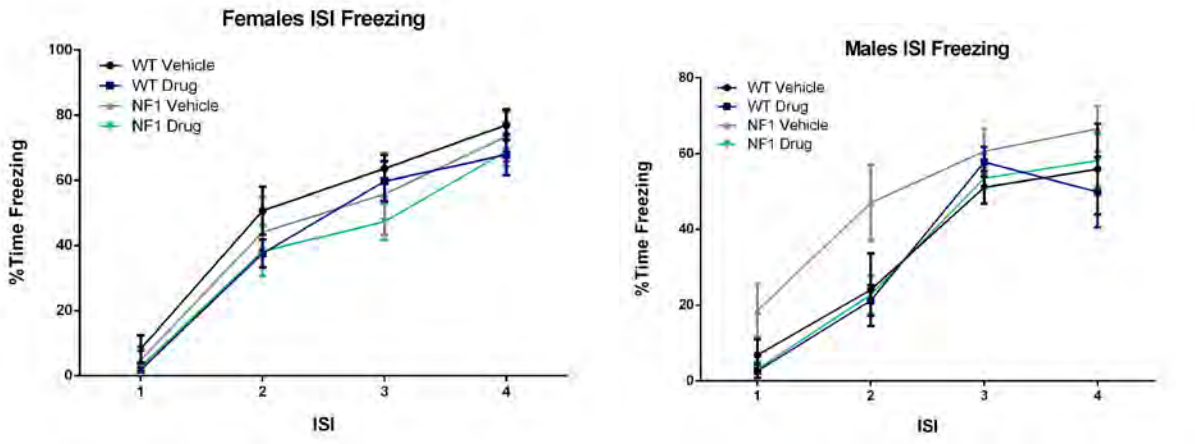

Fig. 8. Effects of Alk inhibitor on freezing between tone-shock pairing on the training day of the contextual fear task. For details, see text.

When the extinction curves for contextual fear memory was analyzed, WT and NF1 mice treated with the Alk inhibitor in general showed lower freezing levels but this did not reach significance (Fig. 9). When individual days were analyzed, there was a treatment x genotype x sex interaction (p = 0.01) and a treatment x interval interaction (p = 0.024) and trends towards an effect of treatment on days 4 (p = 0.074) and 7 (p = 0.080).

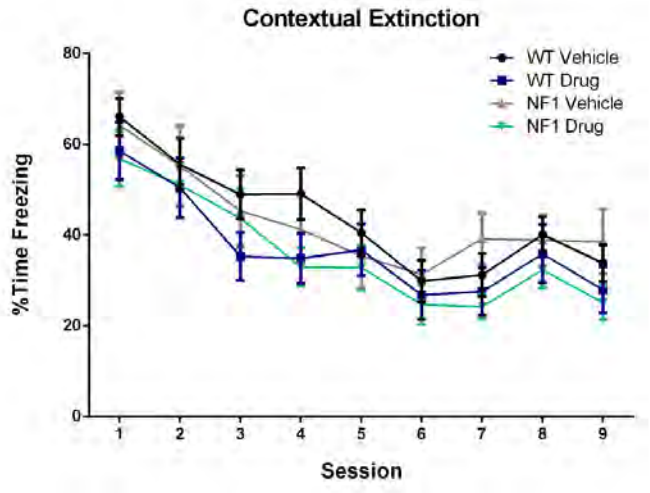

Fig. 9. Effects of Alk inhibitor on extinction of contextual fear. For details, see text.

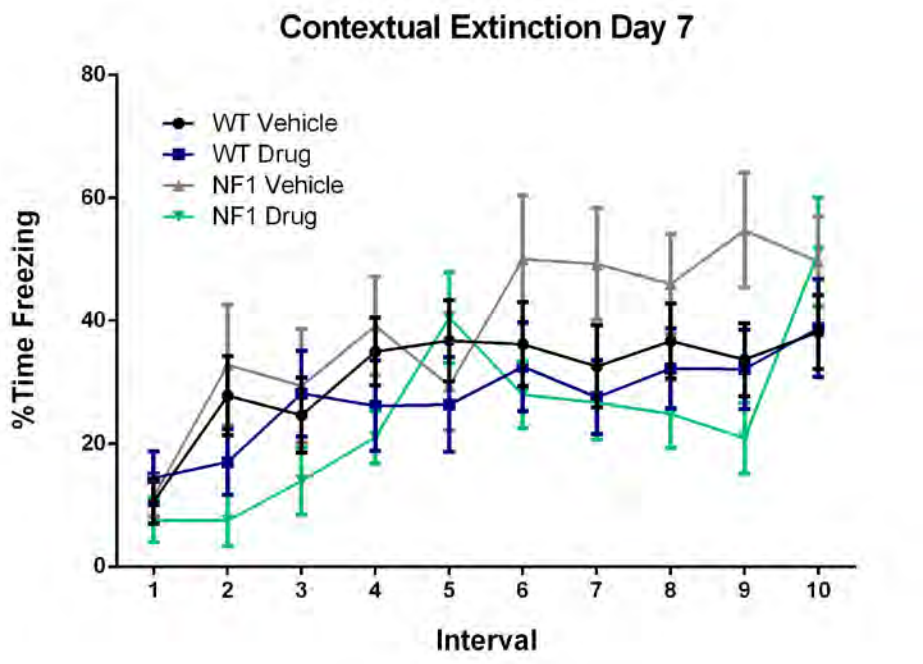

Fig. 10. Effects of Alk inhibitor on extinction of contextual fear on the seventh day of extinction. For details, see text.

Other Achievements

N/A

References

1 Acosta, M. T., Gioia, G. A. & Silva, A. J. Neurofibromatosis type 1: new insights into neurocognitive issues. *Curr Neurol Neurosci Rep* **6**, 136-143 (2006).

2 North, K., Joy, P., Yuille, D., Cocks, N. & Hutchins, P. Cognitive function and academic performance in children with neurofibromatosis type 1. *Dev Med Child Neurol* **37**, 427-436 (1995).

3 Hyman, S. L., Shores, A. & North, K. N. The nature and frequency of cognitive deficits in children with neurofibromatosis type 1. *Neurology* **65**, 1037-1044 (2005).

4 Zoller, M. E., Rembeck, B. & Backman, L. Neuropsychological deficits in adults with neurofibromatosis type 1. *Acta Neurol Scand* **95**, 225-232 (1997).

5 Hofman, K. J., Harris, E. L., Bryan, R. N. & Denckla, M. B. Neurofibromatosis type 1: the cognitive phenotype. *J Pediatr* **124**, S1-8 (1994).

6 Costa, R. M. & Silva, A. J. Molecular and cellular mechanisms underlying the cognitive deficits associated with neurofibromatosis 1. *J Child Neurol* **17**, 622-626; discussion 627-629, 646-651 (2002).

7 Costa, R. M. *et al.* Learning deficits, but normal development and tumor predisposition, in mice lacking exon 23a of Nf1. *Nat Genet* **27**, 399-405 (2001).

8 Silva, A. J. *et al.* A mouse model for the learning and memory deficits associated with neurofibromatosis type I. *Nat Genet* **15**, 281-284, doi:10.1038/ng0397-281 (1997).

9 Klose, A. *et al.* Selective disactivation of neurofibromin GAP activity in neurofibromatosis type 1. *Hum Mol Genet* **7**, 1261-1268 (1998).

10 Gouzi, J. Y. *et al.* The receptor tyrosine kinase alk controls neurofibromin functions in Drosophila growth and learning. *PLoS Genet* **7**, e1002281 (2011).

11 Morris, S. W. *et al.* Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science* **263**, 1281-1284 (1994).

12 Mossâe, Y. P. *et al.* Identification of ALK as a major familial neuroblastoma predisposition gene. *Nature* **455**, 930-935 (2008).

13 Janoueix-Lerosey, I. *et al.* Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. *Nature* **455**, 967-970 (2008).

14 George, R. E. *et al.* Activating mutations in ALK provide a therapeutic target in neuroblastoma. *Nature* **455**, 975-978 (2008).

15 Soda, M. *et al.* Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* **448**, 561-566 (2007).

16 Koivunen, J. P. *et al.* EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. *Clin Cancer Res* **14** (2008).

17 Butrynski, J. E. *et al.* Crizotinib in ALK-rearranged inflammatory myofibroblastic tumor. *N Engl J Med* **363**, 1727-1733, doi:10.1056/NEJMoa1007056 (2010).

18 Kwak, E. L. *et al.* Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* **363**, 1693-1703, doi:10.1056/NEJMoa1006448 (2010).

19 Palmer, R. H., Vernersson, E., Grabbe, C. & Hallberg, B. Anaplastic lymphoma kinase: signalling in development and disease. *Biochem J* **420**, 345-361 (2009).

20 Ishihara, T. *et al.* HEN-1, a secretory protein with an LDL receptor motif, regulates sensory integration and learning in Caenorhabditis elegans. *Cell* **109**, 639-649 (2002).

21 Weiss, J. B., Suyama, K. L., Lee, H. H. & Scott, M. P. Jelly belly: a Drosophila LDL receptor repeat-containing signal required for mesoderm migration and differentiation. *Cell* **107**, 387-398 (2001).

22 Lee, R. C. *et al.* The protocadherin Flamingo is required for axon target selection in the Drosophila visual system. *Nat Neurosci* **6**, 557-563 (2003).

23 Englund, C. *et al.* Jeb signals through the Alk receptor tyrosine kinase to drive visceral muscle fusion. *Nature* **425**, 512-516 (2003).

24 Bazigou, E. *et al.* Anterograde Jelly belly and Alk receptor tyrosine kinase signaling mediates retinal axon targeting in Drosophila. *Cell* **128**, 961-975 (2007).

25 Rohrbough, J. & Broadie, K. Anterograde Jelly belly ligand to Alk receptor signaling at developing synapses is regulated by Mind the gap. *Development* **137** (2011).

26 Cheng, L. Y. *et al.* Anaplastic lymphoma kinase spares organ growth during nutrient restriction in Drosophila. *Cell* **146**, 435-447 (2011).

27 Rohrbough, J., Kent, K., Broadie, K. & Weiss, J. Jelly Belly trans-synaptic signaling to anaplastic lymphoma kinase regulates neurotransmission strength and synapse architecture. *Dev Neurobiol* **73**, 189-208 (2013).

28 Weiss, J. B. *et al.* Anaplastic lymphoma kinase and leukocyte tyrosine kinase: functions and genetic interactions in learning, memory and adult neurogenesis. *Pharmacol Biochem Behav* **100**, 566-574 (2012).